PROCESS FOR PRODUCING CHEESE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims, under 35 U.S.C. 119, U.S. application nos. 60/434,049, filed December 17, 2002 and 60/434,516, filed December 18, 2002, the contents of which are fully incorporated herein by reference

TECHNICAL FIELD

The present invention relates to a process for producing cheese from an enzyme-treated dairy composition.

BACKGROUND OF THE INVENTION

During traditional production of cheese the milk is coagulated by acidification and/or addition of rennet. After coagulation the milk is separated into curd and whey and the whey is drained away from the curd. The cheese is produced by further processing of the curd, whereas the whey is a by-product of the cheese making process. The caseins constitute the major part of the milk protein. The main part of the casein is retained in the curd but some casein, as well as fat, is lost in the whey. Since the cheese is a more valuable product than the whey, there is a desire to reduce the amount of casein and fat lost in the whey and increase the yield of cheese from a volume of milk.

Also, the oiling-off properties of cheese products is an important quality parameter. Oiling-off is the tendency to form free oil upon storage and melting. Excessive oiling-off is a defect most often related to heated products wherein cheese is used, e.g. pizza and related foods (cf. e.g. Kindstedt J.S; Rippe J.K. 1990, J Dairy Sci. 73: 867-873. It becomes more and more important to control/eliminate this defect, as the consumer concern about dietary fat levels increases. Free oil/fat in a product is perceived as a high fat content, and is generally undesirable.

There is a thus a need for improved methods for the manufacture of cheese, in particular methods for improving the cheese yield and/or the stability of the fat in cheese.

WO 00/54601 discloses a method for improving the properties of cheese, including the stability of the fat phase, comprising the steps of a) treating the cheese milk with a phospholipase and b) producing cheese from the cheese milk.

SUMMARY OF THE INVENTION

It has surprisingly been found that when a dairy composition comprising cow's milk and/or one or more cow's milk fractions, is heat treated at a temperature of at least 50°C after or during treatment with phospholipase, and cheese is produced from the treated dairy composition, the oiling off of the cheese is reduced and/or the cheese yield is increased.

The present invention thus relates to a method for producing cheese, comprising: a) treating a dairy composition comprising cow's milk and/or one or more cow's milk fractions, with a phospholipase; b) heat treating said dairy composition at a temperature of at least 50°C; and c) producing cheese from said heat treated dairy composition; wherein step a) is conducted before or during step b).

In another aspect the invention relates to above method further comprising adding calcium to the dairy composition before the heat treatment in step b).

A further aspect of the invention relates to the cheese produced by the methods of the invention.

DETAILED DISCLOSURE OF THE INVENTION

Production of cheese:

In the present context, the term "cheese" refers to any kind of cheese and such as, e.g., natural cheese, cheese analogues and processed cheese. The cheese may be obtained by any suitable process known in the art, such as, e.g., by enzymatic coagulation of a dairy composition with rennet, or by acidic coagulation of a dairy composition with food grade acid or acid produced by lactic acid bacteria growth. In one embodiment, the cheese manufactured by the process of the invention is rennet-curd cheese. Rennet is commercially available, e.g. as

Naturen® (animal rennet), Chy-max® (fermentation produced chymosin), Microlant® (Microbial coagulant produced by fermentation), all from Chr. Hansen A/S, Denmark). The dairy composition may be subjected to a conventional cheese-making process.

Processed cheese is preferably manufactured from natural cheese or cheese analogues by cooking and emulsifying the cheese, such as, with emulsifying salts (e.g. phosphates and citrate). The process may further include the addition of spices/condiments.

The term "cheese analogues" refers to cheese-like products which contain fat (such as, e.g., milk fat (e.g., cream) as a part of the composition, and which further contain, as part of the composition, a non-milk constituents, such as, e.g., vegetable oil.

The cheeses produced by the process of the present invention comprise all varieties of cheese, such as, e.g. Campesino, Chester, Danbo, Drabant, Herregård, Manchego, Provolone, Saint Paulin, Soft cheese, Svecia, Taleggio, White cheese, including rennet-curd cheese produced by rennet-coagulation of the cheese curd; ripened cheeses such as Cheddar, Colby, Edam, Muenster, Gruyere, Emmenthal, Camembert, Parmesan and Romano; blue cheese, such as Danish blue cheese; fresh cheeses such as Mozzarella and Feta; acid coagulated cheeses such as cream cheese, Neufchatel, Quarg, Cottage Cheese and Queso Blanco; and pasta filata cheese. One embodiment relates to the production of pizza cheese by the process of the invention.

In cheese manufacture, the coagulation of a dairy composition is preferably performed either by rennet or by acidification alone resulting in rennet-curd and acid-curd cheese, respectively, making up two major groups of cheese types. Fresh acid-curd cheeses refer to those varieties of cheese produced by the coagulation of milk, cream or whey via acidification or a combination of acid and heat, and which are ready for consumption once the manufacturing without ripening is completed. Fresh acid-curd cheeses generally differ from rennet-curd cheese varieties (e.g. Camembert, Cheddar, Emmenthal) where coagulation normally is induced by the action of rennet at pH values 6.4-6.6, in that coagulation normally occurs close to the isoelectric point of casein, i.e. e.g. at pH 4.6 or at higher values when elevated temperatures are used, e.g. in Ricotta at pH typically about 6.0 and temperature typically about 80°C. In a preferred embodiment of the invention, the cheese belongs to the class of rennet curd cheeses.

Mozzarella is a member of the so-called pasta filata, or stretched curd, cheeses which are normally distinguished by a unique plasticizing and kneading treatment of the fresh curd in hot water, which imparts the finished cheese its characteristic fibrous structure and melting and stretching properties, cf. e.g. "Mozzarella and Pizza cheese" by Paul S. Kindstedt, Cheese: Chemistry, physics and microbiology, Volume 2: Major Cheese groups, second edition, page 337-341, Chapman & Hall. Pizza cheese as used herein includes cheeses suitable for pizzas and they are usually pasta filata/stretched curd cheeses. In one embodiment, the process of the invention further comprises a heat/stretching treatment as for pasta filata cheeses, such as for the manufacturing of Mozzarella.

Dairy composition

A dairy composition according to the invention may be any composition comprising cow's milk constituents. Milk constituents may be any constituent of milk such as milk fat, milk protein, casein, whey protein, and lactose. A milk fraction may be any fraction of milk such as e.g. skim milk, butter milk, whey, cream, milk powder, whole milk powder, skim milk powder. In a preferred embodiment of the invention the dairy composition comprises milk, skim milk, butter milk, whole milk, whey, cream, or any combination thereof. In a more preferred embodiment the dairy composition consists of milk, such as skim milk, whole milk, cream, or any combination thereof.

In further embodiments of the invention, the dairy composition is prepared, totally or in part, from dried milk fractions, such as, e.g., whole milk powder, skim milk powder, casein, caseinate, total milk protein or buttermilk powder, or any combination thereof.

In preferred embodiments, the dairy composition comprises, or consists of, cream. In further embodiments, the dairy composition comprises butter. In still further embodiments, the dairy composition comprises buttermilk.

According to the invention the dairy composition comprises cow's milk and or one or more cow's milk fractions. The cow's milk and cow's milk fractions may be from any breed of cow (*Bos taurus (Bos taurus taurus), Bos indicus (Bos taurus indicus)*, and crossbreeds of these), such as e.g. Ayrshire, Holstein, Friesian, Brown Swiss, Jersey, Milking Shorthorn, Red Dane, Zebu, and Brahma. In one embodiment the dairy composition comprises cow's milk and/or cow's milk fractions originating from two or more breeds of cows.

The dairy composition for production of cheese may be standardised to the desired composition by removal of all or a portion of any of the raw milk components and/or by adding thereto additional amounts of such components. This may be done e.g. by separation of milk into cream and skim milk at arrival to the dairy. Thus, the dairy composition may be prepared as done conventionally by fractionating milk and recombining the fractions so as to obtain the desired final composition of the dairy composition. The separation may be made in continuous centrifuges leading to a skim milk fraction with very low fat content (i.e. e.g. < 0.5%) and cream with e.g. > 35% fat. The dairy composition may be prepared by mixing cream and skim milk. In another embodiment the protein and/or casein content may be standardised by the use of Ultra Filtration.

The dairy composition comprises phospholipids, such as e.g. lecithin. The dairy composition may have any total fat content which is found suitable for the cheese to be produced by the process of the invention, such as, e.g., about 25% fat in dry matter, such as e.g. in the range 10-50% fat in dry matter, of which, e.g., about 0.06% is phospholipids, such as e.g. 0.02-5% (w/w) of the total fat content is phospholipids.

In one embodiment of the invention calcium is added to the dairy composition. Calcium may be added to the dairy composition at any appropriate step before and/or during cheese making, such as before, simultaneously with, or after addition of starter culture. In a preferred embodiment calcium is added before the heat treatment of the dairy composition. In another preferred embodiment calcium is added both before and after the heat treatment. Calcium may be added in any suitable form. In a preferred embodiment calcium is added as calcium salt, e.g. as $CaCl_2$. Any suitable amount of calcium may be added to the dairy composition. The concentration of added calcium will usually be in the range 0.1-5 mM, such as between 1 and 3 mM. If $CaCl_2$ is added to the dairy composition the amount will usually be in the range 1-50 g pr

100 l of dairy composition, such as in the range 5-30 g pr 100 l dairy composition, preferably in the range 10-20 g pr 100 l dairy composition.

Conventional steps may be taken to secure low bacterial counts in the dairy composition. It is generally preferred not to pasteurise skim milk because heat denatured proteins in the dairy composition may have a negative influence on the coagulation, and retard the ripening of the cheese. The bacterial count of skim milk may thus be lowered by other technologies, such as, for example, by microfiltration or bactofugation. Cream is preferably pasteurised to lower the bacterial count in the product. In another preferred embodiment, the dairy composition is raw, unpasteurised milk.

In an embodiment of the invention, the dairy composition may be subjected to a homogenization process before the production of cheese, such as e.g. in the production of Danish Blue Cheese.

The enzymatic treatment:

The enzymatic treatment in the process of the invention may be conducted by dispersing the phospholipase into the dairy composition, and allowing the enzyme reaction to take place at an appropriate holding-time at an appropriate temperature. The treatment with phospholipase may be carried out at conditions chosen to suit the selected enzyme(s) according to principles well known in the art.

The enzymatic treatment may be conducted at any suitable pH, such as e.g., in the range 2-10, such as, at a pH of 4-9 or 5-7.

In one embodiment the phospholipase treatment is conducted at 3-45°C, such as at 25-45°C (e.g., for at least 5 minutes, such as, e.g., for at least 10 minutes or at least 30 minutes, e.g., for 5-60 minutes).

The phospholipase is added in a suitable amount to produce the cheese having the desired properties. Preferably, the phospholipase is added in an amount effective to decrease the oiling-off effect in cheese and/or to increase cheese yield. A suitable dosage of phospholipase will usually be in the range 0.003-0.7 mg enzyme protein per g milk fat, preferably 0.01-0.3 mg enzyme protein per g milk fat, more preferably, 0.03-0.1 mg enzyme protein per g milk fat.

Heat treatment

According to the invention the dairy composition is heat treated after or during phospholipase treatment at a temperature of at least 50°C, such as at least 55°C, preferably at least 60°C, more preferably at least 70°C. In one embodiment the heat treatment is conducted at a temperature between 50°C and 120°C, in a preferred embodiment the heat treatment is conducted at a temperature between 50°C and 100°C, in a more preferred embodiment the heat treatment is conducted at a temperature between 50°C and 80°C. In further embodiments the heat treatment is conducted at temperatures between 60°C and 100°C, such as between 70°C and 90°C. The duration of the heat treatment may be any time suitable to achieve a decrease in oiling off of the cheese and/or an increase in cheese yield. In one embodiment the duration of the heat treatment is between 1 second and 2 hours. The duration of the heat treatment may depend on the heating temperature. In one embodiment the heat treatment is conducted at 50-80°C for 5 seconds-2 hours, in another embodiment the heat treatment is conducted at 80-100°C for 2 seconds-30 minutes, in a still further embodiment the heat treatment is conducted at 100-120°C for 1 second-15 minutes.

If the phospholipase treatment is conducted during the heat treatment, the heat treatment is conducted at a temperature-time combination where the enzyme has a suitable activity to achieve the desired effect within the duration of the treatment.

In one embodiment of the invention the heat treatment is conducted at a temperature-time combination sufficient to inactivate the phospholipase.

The heat treatment may be conducted by any method known in the art, such as e.g. in a plate heat exchanger, by batch wise heating of the milk in a tank or container, or by steam injection.

Enzymes to be used in the process of the invention:

Phospholipids, such as lecithin or phosphatidylcholine, consist of glycerol esterified with two fatty acids in an outer (sn-1) and the middle (sn-2) positions and esterified with phosphoric acid in the third position; the phosphoric acid, in turn, may be esterified to an amino-alcohol. Phospholipases are enzymes which participate in the hydrolysis of phospholipids. Several types of phospholipase activity can be distinguished, including phospholipases A_1 and A_2 (commonly referred to as phospholipase A) which hydrolyze one fatty acyl group (in the sn-1 and sn-2

position, respectively) to form lysophospholipid. Phospholipase B hydrolyzes the remaining fatty acyl group in lysophospholipid.

The enzyme used in the process of the present invention include a phospholipase, such as, phospholipase A_1, phospholipase A_2 and phospholipase B. In the process of the invention the phospholipase treatment may be provided by one or more phospholipase, such as two or more phospholipases, e.g. two phospholipases, including, without limitation, treatment with both type A and B; both type A_1 and A_2; both type A_1 and B; both type A_2 and B; or treatment with two or more different phospholipase of the same type. Included is also treatment with one type of phospholipase, such as A_1, A_2 or B.

Phospholipase A_1 is defined according to standard enzyme EC-classification as EC 3.1.1.32.

Official Name: Phospholipase A_1.

Reaction catalyzed:

phosphatidylcholine + H(2)O <>

2-acylglycerophosphocholine + a fatty acid anion

Comment(s):

has a much broader specificity than EC 3.1.1.4.

Phospholipase A_2 is defined according to standard enzyme EC-classification as EC 3.1.1.4

Official Name: phospholipase A_2.

Alternative Name(s):phosphatidylcholine 2-acylhydrolase.

lecithinase a; phosphatidase; or phosphatidolipase.

Reaction catalysed:

phosphatidylcholine + h(2)o <>

1-acylglycerophosphocholine + a fatty acid anion

comment(s): also acts on phosphatidylethanolamine, choline plasmalogen and phosphatides, removing the fatty acid attached to the 2-position.

Phospholipase B is defined according to standard enzyme EC-classification as EC 3.1.1.5.

Official Name: lysophospholipase.

Alternative Name(s): lecithinase b; lysolecithinase;

phospholipase B; or PLB.

Reaction catalysed:

2-lysophosphatidylcholine + h(2)o <> glycerophosphocholine + a fatty acid anion

Phospholipase A

Phospholipase A activity may be provided by enzymes having other activities as well, such as e.g. a lipase with phospholipase A activity. The phospholipase A activity may e.g. be from a lipase with phospholipase side activity. In other embodiments of the invention phospholipase A enzyme activity is provided by an enzyme having essentially only phospholipase A activity and wherein the phospholipase A enzyme activity is not a side activity.

Phospholipase A may be of any origin, e.g. of animal origin (such as, e.g. mammalian), e.g. from pancreas (e.g. bovine or porcine pancreas), or snake venom or bee venom. Alternatively, phospholipase A may be of microbial origin, e.g. from filamentous fungi, yeast or bacteria, such as the genus or species *Aspergillus,* e.g. *A. niger*; *Dictyostelium,* e.g. *D. discoideum*; *Mucor,* e.g. *M. javanicus, M. mucedo, M. subtilissimus*; *Neurospora,* e.g. *N. crassa*; *Rhizomucor,* e.g. *R. pusillus*; *Rhizopus,* e.g. *R. arrhizus, R. japonicus, R. stolonifer*; *Sclerotinia,* e.g. *S. libertiana*; *Trichophyton,* e.g. *T. rubrum*; *Whetzelinia,* e.g. *W. sclerotiorum*; *Bacillus,* e.g. *B. megaterium, B. subtilis*; *Citrobacter,* e.g. *C. freundii*; *Enterobacter,* e.g. *E. aerogenes, E. cloacae Edwardsiella, E. tarda*; *Erwinia,* e.g. *E. herbicola*; *Escherichia,* e.g. *E. coli*; *Klebsiella,* e.g. *K. pneumoniae*; *Proteus,* e.g. *P. vulgaris*; *Providencia,* e.g. *P. stuartii*; *Salmonella,* e.g. *S. typhimurium*; *Serratia,* e.g. *S. liquefasciens, S. marcescens*; *Shigella,* e.g. *S. flexneri*; *Streptomyces,* e.g. *S. violaceoruber*; *Yersinia,* e.g. *Y. enterocolitica.* Thus, phospholipase A may be fungal, e.g. from the class *Pyrenomycetes*, such as the genus *Fusarium*, such as a strain of *F. culmorum*, *F. heterosporum*, *F. solani*, or a strain of *F. oxysporum*. Phospholipase A may also be from a filamentous fungus strain within the genus *Aspergillus*, such as a strain of *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus niger* or *Aspergillus oryzae*. A preferred phospholipase A is derived from a strain of *Fusarium*, particularly *F. oxysporum*, e.g. from strain DSM 2672 as described in WO 98/26057, especially described in claim 36 and SEQ ID NO. 2 of WO 98/26057. Another preferred phospholipase A is PLA2 from *Streptomyces*, such as e.g. PLA2 from *S. violaceoruber.* In further embodiments, the phospholipase is a phospholipase as disclosed in WO 00/32758 (Novozymes A/S, Denmark).

Phospholipase B

The term "phospholipase B" used herein in connection with an enzyme of the invention is intended to cover an enzyme with phopholipase B activity.

The phospholipase B activity may be provided by enzymes having other activities as well, such as e.g. a lipase with phospholipase B activity. The phospholipase B activity may e.g. be from a lipase with phospholipase B side activity. In other embodiments of the invention the phospholipase B enzyme activity is provided by an enzyme having essentially only phospholipase B activity and wherein the phospholipase B enzyme activity is not a side activity. In one embodiment of the invention, the phospholipase B is not lipases having phospholipase B side activity as defined in WO 98/26057.

The phospholipase B may be of any origin, e.g. of animal origin (such as, e.g. mammalian), e.g. from liver (e.g. rat liver). Alternatively, the phospholipase B may be of microbial origin, e.g. from filamentous fungi, yeasts or bacteria, such as the genus or species *Aspergillus*, e.g. *A. foetidus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. oryzae*; *Botrytis*, e.g. *B. cinerea*; *Candida*, e.g. *C. albicans*; *Cryptococcus*, e.g. *C. neoformans*, *Escherichia*, e.g. *E. coli*, *Fusarium*, e.g. *F. sporotrichioides*, *F. venenatum*, *F. verticillioides*; *Hyphozyma*; *Kluyveromyces*, e.g. *K. lactis*; *Magnaporte*, e.g. *M. grisea*; *Metarhizium*, e.g. *M. anisopliae*; *Mycosphaerella*, e.g. *M. graminicola*; *Neurospora*, e.g. *N. crassa*; *Penicillium*, e.g. *P. notatum*; *Saccharomyces*, e.g. *S. cerevisiae*; *Schizosaccharomyces*, e.g. *S. pombe*; *Torulaspora*, e.g. *T. delbrueckii*; *Vibrio*; e.g. *V. cholerae*. A preferred phospholipase B is derived from a strain of *Aspergillus*, particularly phospholipase LLPL-1 or LLPL-2 from *A. niger*, e.g. as contained in the *Escherichia coli* clones DSM 13003 or DSM 13004, or phospholipase LLPL-1 or LLPL-2 from *A. oryzae*, e.g. as contained in the *E. coli* clones DSM 13082 or DSM 13083 as described in WO 01/27251, especially described in claim 1 and SEQ ID NOs. 2, 4, 6 or 8 of WO 01/27251.

Enzyme sources and formulation

The phospholipase used in the process of the invention may be derived or obtainable from any of the sources mentioned herein. The term "derived" means in this context that the enzyme may have been isolated from an organism where it is present natively, i.e. the identity of the amino acid sequence of the enzyme are identical to a native enzyme. The term "derived" also means that the enzymes may have been produced recombinantly in a host organism, the recombinant produced enzyme having either an identity identical to a native enzyme or having a modified amino acid sequence, e.g. having one or more amino acids which are deleted, inserted and/or substituted, i.e. a recombinantly produced enzyme which is a mutant and/or a fragment of a native amino acid sequence. Within the meaning of a native enzyme are included natural

variants. Furthermore, the term "derived" includes enzymes produced synthetically by e.g. peptide synthesis. The term "derived" also encompasses enzymes which have been modified e.g. by glycosylation, phosphorylation etc., whether in vivo or in vitro. The term "obtainable" in this context means that the enzyme has an amino acid sequence identical to a native enzyme. The term encompasses an enzyme that has been isolated from an organism where it is present natively, or one in which it has been expressed recombinantly in the same type of organism or another, or enzymes produced synthetically by e.g. peptide synthesis. With respect to recombinantly produced enzyme the terms "obtainable" and "derived" refers to the identity of the enzyme and not the identity of the host organism in which it is produced recombinantly.

Accordingly, the phospholipase may be obtained from a microorganism by use of any suitable technique. For instance, a phospholipase enzyme preparation may be obtained by fermentation of a suitable microorganism and subsequent isolation of a phospholipase preparation from the resulting fermented broth or microorganism by methods known in the art. The phospholipase may also be obtained by use of recombinant DNA techniques. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector comprising a DNA sequence encoding the phospholipase in question and the DNA sequence being operationally linked with an appropriate expression signal such that it is capable of expressing the phospholipase in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may also be incorporated into the genome of the host cell. The DNA sequence may be of genomic, cDNA or synthetic origin or any combinations of these, and may be isolated or synthesized in accordance with methods known in the art.

Suitable phospholipases are available commercially. As typical examples of the enzymes for practical use, pancreas-derived phospholipase A_2 such as Lecitase® (manufactured by Novozymes A/S, Bagsværd, Denmark) is preferably used. A suitable phospholipase B is e.g. *Aspergillus niger* phospholipase LLPL-2 that can be produced recombinantly in *A. niger* as described in WO 01/27251.

In the process of the invention the phospholipase may be purified. The term "purified" as used herein covers phospholipase enzyme protein free from components from the organism from which it is derived. The term "purified" also covers phospholipase enzyme protein free from components from the native organism from which it is obtained, this is also termed "*essentially*

pure" phospholipase and may be particularly relevant for phospholipases which are naturally occurring and which have not been modified genetically, such as by deletion, substitution or insertion of one or more amino acid residues.

Accordingly, the phospholipase may be purified, viz. only minor amounts of other proteins being present. The expression "other proteins" relate in particular to other enzymes. The term "purified" as used herein also refers to removal of other components, particularly other proteins and most particularly other enzymes present in the cell of origin of the phospholipase. The phospholipase may be "substantially pure", i.e. free from other components from the organism in which it is produced, i.e., e.g., a host organism for recombinantly produced phospholipase. Preferably, the enzymes are at least 75% (w/w) pure, more preferably at least 80%, 85%, 90% or even at least 95% pure. In a still more preferred embodiment the phospholipase is an at least 98% pure enzyme protein preparation. In other embodiments the phospholipase is not naturally present in milk.

The terms "phospholipase" includes whatever auxiliary compounds that may be necessary for the catalytic activity of the enzyme, such as, e.g. an appropriate acceptor or cofactor, which may or may not be naturally present in the reaction system.

The phospholipase may be in any form suited for the use in question, such as e.g. in the form of a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a protected enzyme. Granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be stabilized by adding stabilizers such as a sugar, a sugar alcohol or another polyol, lactic acid or another organic acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

By the process of the invention, the lecithin content of the cheese may be reduced by at least 5%, such as at least 10%, at least 20%, at least 30%, at least 50%, such as in the range of 5-95% compared to a similar cheese making process but without the enzymatic treatment of a phospholipase, as described herein.

In cow milk, the lecithin constitutes normally more than 95% of the phospholipids in milk whereas the lysolecithin is approximately 1% of the phospholipids. Although the phospholipids

represent normally less than 1% of the total lipids in cow milk, they play a particularly important role, being present mainly in the milk fat globule membrane. By the process of the present invention the lecithin content in the obtainable cheese may be less than 90%, such as e.g. less than 80%, e.g. less than 60% or less than 50% of the total content of phospholipid in the cheese. The lecithin content may be measured by any method known by the skilled person, e.g. by HPLC.

The present invention further relates to use of the cheese produced by the process of the invention in pizza, ready-to-eat dishes, processed cheese, or as an ingredient in other food products. Accordingly, the cheese produced according to the process of the invention may be used in further processed food products like processed cheese, pizza, burgers, toast, sauces, dressings, cheese powder or cheese flavours.

In further embodiments, the process of the invention further comprises the step of subjecting the cheese to a heating treatment, such as, e.g., in the range 150-350°C.

The invention also relates to a cheese obtainable, in particular obtained, by the process of the invention.

The present invention is further illustrated in the following examples which are not to be in any way limiting to the scope of protection.

Example 1

Depletion of phospholipids in cream

Cream was treated with two different amounts of a phospholipase to determine the depletion of phospholipids by the action of the enzyme.

Substrate Preparation and Enzyme/Substrate Reaction.

Cream was standardized to a fat content of 25% (w/w) using skim milk. Two samples of 1 ml each were incubated with 0.02 mg enzyme protein and 0.1 mg enzyme protein per gram of milk fat, respectively, of a phospholipase (Lecitase®, Novozymes A/S, Bagsværd, Denmark) at 35°C

for 1.5 hr without shaking. Reactions were stopped by the addition of organic solvent for lipid extraction.

Lipid Extraction.

Total milk lipids were extracted by mixing each sample with 1 ml of water followed by 9 ml of chloroform/methanol (2:1). Samples were mixed vigorously for 1 min and centrifuged at 3000 rpm for 5 min. Six milliliters of the lower organic phase were removed and dried down under vacuum. Samples were reconstituted in 2 ml of chloroform. Each chloroform extract was applied to an aminopropyl SPE column (Phenomenex, Inc.) under vacuum. The column was washed first with 4 ml of chloroform/isopropanol (2:1) to remove neutral lipids and then with with 4 ml of diethylether acidified with glacial acetic acid (2% v/v) to remove free fatty acids. Phospholipids were then eluted with 4 ml of methanol, dried down in a rotary evaporator, and reconstituted in 0.6 ml of mobile phase A for HPLC analysis.

HPLC determination of phosphatidylethanolamine (PE)

HPLC was performed on a Agilent 1100 system containing a quaternary pump, degasser, autosampler, thermostated column compartment, evaporative light scattering detector (Polymer Laboratories, Inc.), and a personal computer with Agilent ChemStation software. The stationary phase consisted of a Luna Silica (150 x 4.6 mm, 5 μ, 100 A) analytical column and a Security Guard Cartridge (4.0 x 3.0 mm) consisting of the same packing material. Both analytical and guard columns were from Phenomenex (Torrance, CA USA). The mobile phases consisted of an A mixture containing 80% chloroform, 19.5% methanol, 0.5% ammonium hydroxide and a B mixture of 60% chloroform, 34% methanol, 5.5% water, 0.5% ammonium hydroxide. The following linear gradient was utilized: a starting composition of 80% A/20% B was held for 2 min, proceeding to 100% B from 2 min to 14 min; 100% B was maintained from 14 min to 20 min, returning to 80% A/20% B from 20 min to 23 min. The time required to re-equilibrate the column in a sequence of runs was 7 min. With a flow rate of 1.0 ml/min and a column temperature of 30°C, the pressure increased from approximately 43 to 55 bar. The evaporator temperature of the light scattering detector was 80°C and the nebulizer temperature was 42°C. The nebulization gas was nitrogen used at a flow rate of 1.0 SLM. Highly pure phosphatidylethanolamine (PE) (Avanti Polar Lipids, Inc.) was used as standard. Stock solution was prepared in chloroform in the concentration range of 2-10 mg/ml. HPLC calibrators were prepared from stock solutions by dilution to the appropriate concentration in mobile phase A.

Table 1. Results of example 1

Enzyme dose (mg enzyme protein/g fat)	% PE Depletion (versus control without phospholipase treatment)
0.02	80%
0.10	>90% *

*Peak below lowest limit of quantification

Example 2

Production of Mozzarella cheese from cow's milk

Pasteurized, non-homogenized cream (North Carolina State University Dairy Plant) was used to standardize five hundred grams pasteurized, non-homogenized skim milk (North Carolina State University Dairy Plant) to 3.5% fat thus producing full fat mozzarella cheese.

The cheese milk (made above) was then treated in 3 different ways and cheeses were prepared as described below. For each treatment a control cheese was prepared in the same manner except that phospholipase was not added.

1. No heat treatment - phospholipase added with rennet

The cheese milk was placed in a 35°C water bath until equilibrated to that temperature. While cheese milk was equilibrating, starter culture was prepared by adding 0.18 grams each of Rhodia LH100 and TA061 cheese cultures (Rhodia, Wisconsin, USA) into 250 ml skim milk and tempered at 35°C for 30 minutes. The initial pH of the cheese milk was taken and 20 ml starter was transferred into each beaker and stirred at 35°C.

pH was monitored until a pH of 6.4 was reached. One ml rennet (Novozym 89L), which was diluted to 250 μl enzyme in 9 ml total solution with deionized water and Lecitase® (0.5 mg/(g fat)), were each added separately and stirred vigorously for 3 minutes. The stir bar was removed and the rennetted milk was allowed to sit at 35°C.

2. No heat treatment – phospholipase added with cheese culture

The cheese milk was treated with 0.5 mg/(g fat) Lecitase® (Novozymes A/S, Bagsværd, Denmark) and placed in a 35°C water bath until equilibrated to that temperature. The initial pH

of the cheese milk was taken and 20 ml starter prepared as above was transferred into each beaker and stirred at 35°C.

pH was monitored until a pH of 6.4 was reached. One ml rennet (Novozym 89L), which was diluted to 250 μl enzyme in 9 ml total solution with deionized water was added and stirred vigorously for 3 minutes. The stir bar was removed and the rennetted milk was allowed to sit at 35°C.

3. Heat treatment at 50°C – phospholipase added before heat treatment

The cheese milk was treated with 0.5 mg/(g fat) Lecitase® and incubated at 50°C for 30 minutes. After incubation the cheese milk was placed in a 35°C water bath until equilibrated to that temperature. The initial pH of the cheese milk was taken and 20 ml starter prepared as above was transferred into each beaker and stirred at 35°C.

pH was monitored until a pH of 6.4 was reached. One ml rennet (Novozym 89L), which was diluted to 250 μl enzyme in 9 ml total solution with deionized water was added and stirred vigorously for 3 minutes. The stir bar was removed and the rennetted milk was allowed to sit at 35°C.

After the above treatments, curd was ready to cut when a spatula was inserted and sharp edges were seen. The cheese was cut by pushing the cutter down and while holding the beaker quickly turning the cutter and finally pulling the cutter up. The curd was allowed to rest for 3-5 minutes then stirred gently with spoon. The curd was drained for 5 minutes using funnel and cheesecloth. Curd was placed in a stainless steel bowl and allowed to float in a 41°C water bath pouring off whey as needed.

When the curd reached pH 5.3, the stainless steel bowl with the curd in it was flooded in a water bath at 57°C for 5 minutes. The curd was then hand stretched, replacing in water bath when necessary to return curd to 57°C. Curd was tempered in cold water for 10-15 minutes. The cheese curd was weighed and refrigerated overnight.

Measurement of Oiling Off

The diffusion of fat/oil was tested on cheese samples heated in the oven at 90°C for 5 minutes. Before the diffusion test, the cheese was ground in a blender for 20 seconds for uniformity of sample. Then 3.0 grams were molded into a metal ring (2.2 cm) and placed in the center of a Whatman #4 filter paper. Oiling off was determined by image analysis, difference in the areas between the ring of oil and the circle of cheese. The calculation for oiling off follows:

$$\text{Ratio of Oiling Off} = \frac{(\text{Total Area} - \text{Area of Cheese}) \times 100}{\text{Area of Cheese}}$$

Measurement of Meltability

Meltability was tested on cheese samples heated in the oven at 100°C for 14 minutes. Before the meltability test, the cheese was ground in a blender for 20 seconds for uniformity of sample. 3.0 grams was then molded into a metal ring (2.2 cm) and placed in the center of a glass petri dish. Meltability was determined by calculating area of the cheese before and after melting. The calculation for meltability was as follows:

$$\text{Ratio of Meltability} = \frac{(\text{Area After Melt} - \text{Area Before Melt}) \times 100}{\text{Area Before Melt}}$$

Results

Table 2. Results of cheesemaking experiments

Treatment	**Meltability** % of control	**Oiling off** % of control
1 (no heat treatment – phospholipase added with rennet)	115	94
2 (no heat treatment – phospholipase added with cheese culture)	107	96
3 (heat treatment at 50°C – phospholipase added before heat treatment)	106	45

From the results in table 2 it is seen that all three treatments lead to a small increase in meltability. The largest reduction in oiling-off was clearly achieved with treatment 3 wherein

phospholipase was active during the heat treatment. The effect cannot be explained by increased enzyme activity during heat treatment since comparing with example 1 it is clear that with the present enzyme concentration the reaction had run to completion even when no heat treatment was performed.

Example 3

Production of Mozzarella cheese from cow's milk

Mozzarella cheese can be produced according to the invention in the following way:

Cow's milk is standardised to 3% fat by mixing skim milk (0.06% fat) and cream (40.0% fat).

Two milk batches are designed as controls (no phospholipase treatment) and two batches are treated with phospholipase enzyme for 1 hour at 35°C. The optimal enzyme dosage can be determined by any method known in the art, e.g. by testing a number of different enzyme dosages within the ranges given in the description of the present application.

One control and one enzyme treated batch are pasteurized in water-bath at 63°C for 30 min, then placed in ice-water to cool down to 35°C. 0.02% of 40% $CaCl_2$ solution is added to the pasteurized samples.

A starter culture, e.g. 0.01% of a mixture of starter cultures TA 061 and LH 100 (Chr. Hansen, Milwaukee, WI), and rennet, e.g. 0.09% w/w of Chymax (Chr. Hansen, Milwaukee, WI), is added to each batch of cheese milk, and the cheese milk is left for 1 hour at 35°C.

After coagulation the coagulum is cut with 0.5" wire cutters.

The cheese curd is processed after a conventional protocol for Mozzarella cheese.

Actual cheese yield can be calculated as the weight of cheese after stretching relative to the total weight of cheese.

Moisture adjusted cheese yield is expressed as the actual yield adjusted to standard constant

level of moisture. Moisture adjusted yield is calculated by multiplying the actual yield and the ratio of actual moisture content to standard moisture, according to the following formula:

$Y_{adj} = Y_{act} \times 1- M_{act} / 1-M_{std}$

where Y_{adj} = moisture adjusted yield, Y_{adj} = actual yield, M_{act} = actual moisture fraction & M_{std} = standard moisture fraction, e.g. 0.48

Oiling-off is measured as described in Example 1.

The effect of phospholipase treatment can be evaluated by comparison of cheese yield and oiling off between cheese made of phospholipase treated milk and control cheese.